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54 **Gonococcal and meningococcal polypeptides, vaccines and diagnostics.**

57 The subject matter of the invention is a polypeptide which includes an amino acid residue sequence constituted by at least 5 and up to about 80 amino acid residues, and which is capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.

The polypeptide of the invention can be used as a vaccine or diagnostic for the prevention of gonorrhea and/or meningitidis.

**EP 0 273 116 A2**

A vaccine or diagnostic composition and a polypeptide useful therefor

This invention concerns a vaccine or diagnostic for the prevention of gonorrhea and/or meningitis, utilizing antigenic and immunogenic parts of a protein or utilizing antibodies raised by such immunogens.

*Neisseria gonorrhoeae*, a gram-negative coccus, is the causative agent of the human sexually transmitted disease gonorrhea. In men, the disease is usually symptomatic in 90% of the cases. Without treatment, males may suffer local extension of the infection to the prostate and accessory glands, which may be an important cause of male infertility. In contrast, in 50% of infected women, symptoms are absent or not severe. In 10-15% of the women, the infection extends to the fallopian tubes, causing Salpingitis, ectopic pregnancy, infertility and pelvic inflammatory disease. In 1-3% of the cases of men or women, a systemic invasion by the organism can occur, causing arthritis, endocarditis and disseminated infection. But with adequate treatment, the morbidity is slight. (Davis et al., In: Microbiology; Harper International Edition, 1980).

*Neisseria meningitidis* is the major agent of bacterial meningitis. The organisms usually inhabit the human nasopharyngeal area without any symptoms, enhancing the immunity of the host. When individuals without adequate levels of immunity acquire the meningococcus, the resulting nasopharyngeal infection may lead to Bacteremia, ordinarily followed by acute meningitis. Despite treatment of meningitis infections, case fatality rates are about 10%, and neurological sequelae are common (Davis et al., loc. cit.).

Despite the widespread use of antibodies, diseases caused by *N. gonorrhoeae* and *N. meningitidis* continues to be a serious problem in both developing and industrialized countries, due to the rise of penicillinase-producing strains. Gonorrhea vaccines based on the use of whole cells have been employed a number of times, but were not efficient (Greenburg et al., "Preliminary Studies On the Development Of A Gonococcal Vaccine"; Bull. Wld. Org. 45, 531, 1971). Meningitis vaccines consisting of capsular polysaccharides seem to be more suitable for epidemic control than for treatment.

In order to develop a more efficient vaccine or diagnosticum based on purified components, several investigations have been concentrated on surface antigens of *N. gonorrhoeae* and *N. meningitidis*, including lipopolysaccharides (LPS), peptidoglycan, IgA1 protease, pili and outer membrane proteins. The international application WO85/04654 proposes immunogenic pilus peptides encompassing two conserved regions within the predominantly variable C-terminus. These defined antigenic determinant sites are thought to be useful to prepare peptide vaccines. Due to the complexity of immunological reactions there, however, continues to exist a considerable need for the determination and provision of further antigenic determinant sites to amplify the scope and specificity of the vaccines or diagnostics.

It is an object of the present invention to provide a polypeptide and the pharmaceutically acceptable salts thereof which is capable of being an antigen, or an immunogen, against a gonococcal and/or meningococcal infection and which amplifies the scope and specificity of vaccines or diagnostics.

It is a further object of the present invention to provide diagnostic compositions comprising a polypeptide as mentioned above which are suitable for the detection of antibodies in a sample such as blood-serum of a patient.

An additional object of the present invention is to provide a diagnostic composition suitable for the detection of a gonococcal opacity protein (Protein II) and/or a meningococcal class 5 protein.

Furthermore it is an object of the present invention to provide a vaccine composition suitable for the prevention of gonorrhea and/or meningitis.

Still further it is an object of the present invention to provide a genetically modified bacterial or viral system which comprises a live vaccine suitable for the prevention of gonorrhea and/or meningitis and which elicits at least one of the above mentioned polypeptides.

Finally it is an object of the present invention to provide a method of immunizing a patient in which the vaccine composition or the genetically modified bacterial or viral systems mentioned above are administered or delivered via a suitable route, respectively.

According to the present invention, the solution of the above technical problems essentially is achieved by providing new conserved regions of the opacity protein (Op protein), also referred to as Protein II. On the surface of intact gonococci Op proteins can be assigned to serological classes with little antigenic relatedness, although these proteins share common peptides reminiscent of the constant regions of the pilus protein (Swanson and Barrera, J. Exp. Med. 157, 1405-1420, 1983; for detailed description see: Stern et al., Cell 37, 447-456, 1984). Most of the constant portions, revealed for the gonococcal opacity proteins, could be recovered in the meningococcal class 5 proteins. Apart from the constant regions, the class 5 protein of *N. meningitidis* share some additional characteristics with the opacity proteins of *N. gonorrhoeae*: (a) location in the outer membrane, (b) temperature-modifiable mobility in SDS polyacrylamide gels, (c)

inter-strain and intra-strain variability of amino acid composition restricted to distinct areas of the proteins (Frasch and Mocca, J. Bacteriol., 78, 1127-1134, 1978; Zollinger and Mandrell, Infect. Immun. 28, 451-458, 1980; Judd, Infect. Immun. 48, 452-457, 1985).

Investigations have revealed that the colonization of gonococci in the human mucosa, the initial step of the infection, appears to be dependent on the expression of the pilus and the opacity protein. Preliminary studies suggest that the mechanisms of attachment to the mucosa by *N.gonorrhoeae* and *N.meningitidis* are similar or possibly identical (McGee et al., Rev. Infect. Dis. 5, 708-714, 1983). To complete a vaccine composed of constant pilus-specific peptides and/or to develop a diagnostic suitable for the pathogenic species *N. gonorrhoeae* and *N. meningitidis*, it was looked for conserved antigenic determinants that can be used for the preparation of antigens and/or immunogens that correspond to these antigenic determinants and that enhance antibody production in the host.

The polypeptide of this invention is capable of immunologically mimicking a conserved antigenic site of the opacity protein in *N.gonorrhoeae* and/or the class 5 proteins in *N.meningitidis*. It comprises at least one peptide of at least 5 amino acid residues up to about 2000 amino acid residues, that defines an immunogenic and antigenic site of a gonococcal Op protein and/or meningococcal class 5 proteins. Such a polypeptide is represented by an amino acid sequence of at least 5 up to about 80 amino acid residues that can be repeated as a unit one or more times in the same polypeptide molecule, or more than one type of such repeating unit can be present in a single polypeptide molecule. For further explanation see definition of the term "polypeptide", below.

Such polypeptide can be made as a fusion protein constructed by genetic engineering techniques or it can be synthesized from individual amino acid residues.

The corresponding DNA sequences can be either obtained from natural sources or they can be obtained by DNA synthesis according to known methods.

Polypeptides embodying this invention are deduced from the sequences of opacity proteins and/or class 5 proteins, summarized in Figure 1.

The term "polypeptide", as described herein, refers to a proteinous molecule that, as a whole, not typically occurs in nature, but results from intellectual and technical effort. Such a polypeptide preferentially consists of L-amino acid residues typically found in nature, however, chemical modifications of naturally-occurring amino acid residues may also be included in case they are capable of mimicking an antigenic determinant site. The polypeptides, as described herein, may be constituted by at least five amino acid residues and up to about 2000 amino acid residues. Typically such a polypeptide can be produced by chemical means (e.g., see Merrifield, J. Am. Chem. Soc. 85, 2149-2154, 1963) or by microorganisms which have been genetically engineered (e.g. see Silhavy et al., Microbiol. Rev. 47, 313-344, 1983). Polypeptides produced in that way should include at least one of the amino acid sequences described herein. The polypeptides also may include multiple identical sequences, and/or in conjunction with other amino acid sequences as described herein, and/or in conjunction with unrelated amino acid sequences.

"Amino acid sequences", as described herein, may represent at least five and up to about 80 amino acid residues of a continuous amino acid sequence of a gonococcal opacity protein and/or a meningococcal class 5 protein. The amino acid sequences, as described herein, should resemble at least one conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or a meningococcal class 5 protein.

The term "antigenic determinant", as used herein, refers to a structural element of a polypeptide which is capable of interacting with a corresponding receptor molecule. A "conserved" antigenic determinant site, as described herein, is an amino acid sequence widely common to opacity proteins and meningococcal class 5 proteins, thus capable of binding to an identical receptor molecule, respectively. Figure 1 summarizes the DNA/protein sequences of 6 opacity proteins and 1 class 5 protein, providing a basis for this invention.

The phrase "immunologically mimicking" is used herein to mean that the polypeptides of this invention are not naturally occurring proteins or proteolytic products thereof. Furthermore, this phrase also includes the possibility that an "antigen related variant" of a given polypeptide is capable of binding to, and/or of eliciting the production of, an identical receptor molecule.

Multiple evidence for the existence of such antigenically related variants has been summarized recently; see WO85/04654, pp. 9-12. Data obtained from immuno assays and from studies of protein-protein interactions in solved X-ray crystallographic structures indicates that the protein-protein recognition does not require a strict conservation of the amino acid sequence for relatedness. A single amino acid residue exchange can influence the structure of a given polypeptide in different ways depending upon the nature of the substitution. The degree of relatedness of two different amino acid sequences can be expressed in terms of several amino acid parameters.

In this invention two polypeptides should be considered as two antigenically related variants provided that the confidence level of these amino acid parameters is not less than 95 %, or preferably, greater than 99 %.

The term "receptor molecule", as used in this invention, refers to a molecule to which a polypeptide, and an antigenically related variant thereof, carrying an antigenic determinant site can bind with high specificity. Such receptor molecules can be elicited by using a polypeptide as an immunogen in an animal host, preferentially a mammal. For this purpose the immunogen will be administered in a suitable form with respect of the carrier, the adjuvant, the dose, and the site of application, etc. The resulting immunoglobulines, i.e. the receptor molecules, as well as parts of the receptor molecules still capable of binding to the antigenic determinant site, i.e. their immunoglobuline combining sites, can be used for diagnostic purposes. Such receptor molecules might also be useful for passive protection against gonococcal infection.

A "diagnostic composition" includes one or more such receptor molecules specific for antigenic determinant sites in one or more polypeptides described in this invention. In addition, a diagnostic composition according to this invention includes the means, such as labelled secondary antibody, necessary to detect a specific reaction of a receptor molecule with its corresponding antigenic determinant site in a gonococcal opacity protein (protein II) and/or a meningococcal class 5 protein.

In humans the polypeptides described in this invention can be effective in preventing an infection with gonococci and/or meningococci. Two principal strategies can be chosen for an immunization against gonococcal and/or meningococcal infection using polypeptides described in this invention:

(a) A "vaccine composition" containing at least one polypeptide of this invention in combination with a suitable and pharmaceutically acceptable adjuvant and/or diluent. In case the polypeptide(s) used in this vaccine composition are not immunogenic by themselves, which is usually true for polypeptides containing less than about 20 amino acid residues, the polypeptide is coupled to a suitable carrier molecule. A typical coupling reaction is described in Klipstein et al., J. Infect. Dis. 147, 318, 1983.

For immunizing a patient against gonorrhea and/or meningitis such a peptide vaccine composition will be administered in a suitable form by injection, orally or nasopharyngeally. The effective amounts of a polypeptide that is administered to a human by this method range from about 10 micrograms to about 100 milligrams without carrier.

(b) The peptides of this invention can be elicited by genetically modified living bacteria or by cells of a patient after infection with a genetically modified virus, thus constituting a live vaccine that can be used as an alternative method of immunizing a patient against gonorrhea and/or meningitis.

A genetically modified bacterial system suitable for this purpose typically consists of an attenuated bacterial host, in which expressable gene sequences have been incorporated capable of expressing (a) polypeptide(s) of this invention. The bacterial host modified in this way is capable of transiently (usually up to about 14 days) colonizing a mucosal site of the patient and, by delivering polypeptides of this invention, induces mucosal immunity against gonorrhea and/or meningitis. A typical bacterial host used for this purpose is *Salmonella typhi* Ty21a (Formal et al., Infect. Immun. 34, 746-750, 1981), but other suitable bacterial strains can be used too. Polypeptides of this invention are typically produced by the bacterial host as fusion proteins (Young et al., PNAS USA, 80, 1194-1198, 1984) that can be accumulated in, or released from, the cytoplasm of the bacterial host. The living bacteria are inoculated on mucosal surfaces of the patient in typical amounts of up to about  $10^{12}$  colony forming units per individual. When administered orally the live bacterial vaccine is preferentially given in capsules in combination with neutralizing reagents.

A genetically modified viral system typically involves an attenuated vaccinia virus (see Brown et al., Nature 319, 549-550, 1986). For the purposes described in this invention, the genome of such a modified vaccinia virus contains the coding sequences for one or more polypeptides as described herein. Upon infection of the cells of a patient the viral vaccine gives rise to the production of polypeptides according to this invention. For immunizing a patient with a live viral vaccine of this invention up to about  $10^{11}$  plaque-forming units are inoculated per individual, with preference intradermally or subcutaneously.

The Figures show:

Figure 1:

Comparison of the DNA and the amino acid sequences of 6 complete gonococcal opacity proteins (NG1-NG6) and 1 meningococcal class 5 protein (NM1).

- 5 The sequences were aligned using a computer program. All polypeptides corresponding to the invention are marked by frames.

Figure 2:

- 10 Distribution of hydrophilic and hydrophobic amino acid sequences within opacity and class 5 proteins exemplified by the hydropathy plot of the gonococcal protein NG2. In the plot the values for the leaderpeptide are included; therefore, the number of the amino acid positions do not correlate to the numbers used in Figure 1 and Table 1, respectively. Positive values refer to hydrophilic amino acid  
15 residues, negative values to hydrophobic amino acid residues. The locations of amino acid sequences selected for the synthesis of polypeptides are marked by arrows.

#### Example 1: Construction of Fusion Proteins Suitable for Diagnostic and Vaccine Purposes

- 20 Polypeptides of interest can be expressed as a portion of fusion proteins. For this purpose, defined segments of the cloned opacity gene including the coding regions for the conserved antigenic determinant sites are expressed in E.coli as fusion to the N-terminal part of the MS2-polymerase gene under the control of the inducible PL promoter (Strebel et al., J. Virol. 57, 983-991, 1986). But other expression systems  
25 yielding high amounts of fusion proteins can be used, too. First of all, it is necessary to enclose the opacity gene segments that encode the conserved antigenic determinant sites. Therefore, we constructed a series of Bal 31 derivatives of the opacity gene (NG1), truncated at the 5' end. After exonucleolytic digestion for different stretches, the truncated gene segments are subsequently fused to the MS2-polymerase coding part of the expression vector pEX. Thus the resulting fusion proteins harbour a 12 kd portion of the  
30 bacteriophage MS2-polymerase at their amino termini and opacity-specific sequences of different length at their carboxy termini. All constructs yield large amounts of proteins. Purification of fusion proteins is done as described by Strebel et al.(loc.cit.) A general explanation of the methods applied in carrying out the present invention can be found in Maniatis et al., "Molecular Cloning", CSH Laboratory, Cold Spring Harbor, New York (1982).

#### Example 2: Immunization

- 40 Rabbits can be utilized herein to raise anti-fusion protein antibodies. Therefore, the host animal is primed with about 150 µg of the purified fusion protein suspended in Freund's complete adjuvant. The rabbits are typically injected subcutaneously with the first inoculum. The rabbits are boosted at intervals of 10-14 days with the same amount of protein in Freund's incomplete adjuvant; therefore, the rabbits are injected intraperitoneally on day 10, 20 and 30, respectively, of the immunization schedule. Each immuniza-  
45 tion consists of four injections of the inoculum. Mice may be immunized in a similar way using about one tenths of the mentioned dose per injection.

Animals are typically bled 10 days after the last injection. Control pre-immune serum is obtained from each animal by bleeding just before the initial immunization.

Sera are titrated by enzyme-linked immunosorbent assay (Bittle et al., Nature 298, 30-33, 1983) with the purified antigen (used for immunization) as well as unpurified fusion proteins (total cell extract).

- 50 If desired, cross-reaction between the antisera due to the N-terminal MS2-specific sequences shared by all fusion proteins, could be eliminated by competing with an extract of E.coli cells expressing the MS2-polymerase fragment exclusively. Alternatively, control inoculum solutions can be prepared using the MS2-polymerase expressed by such E.coli strains.

### Example 3: Western Blot Assay

In a Western blot assay the rabbit antisera are investigated for their ability to immunoreact with gonococcal opacity proteins and meningococcal class 5 proteins, respectively. For immunoblotting of these proteins from cell lysates, colonies are grown on agar plates and collected. Cells equivalent to 2 mg of total protein are suspended in 150  $\mu$ l sample solution (Laemmli, Nature 227, 680-685, 1970) and homogenized by sonication. After boiling, an aliquot of 15  $\mu$ l is loaded on a SDS-polyacrylamid gel and electrophoresed. The proteins are transferred from the gel onto nitrocellulose sheets at 1V/cm<sup>2</sup> (Blake et al., Anal. Biochem. 136, 175-179).

The rabbit anti-fusion protein antisera are diluted approximately 1:100 in 3% BSA/PBS. The nitrocellulose filter with the bound antigen is incubated in the diluted antisera overnight. The filters are washed repeatedly in 0.5% Tween/TBS, once in TBS, soaked in TBS/BSA for 5 min., and incubated in a solution containing protein A coupled to alkaline phosphatase (in TBS/BSA) at room temperature for 1 hour. The filters are washed as described previously and incubated in a 5-bromo-4-chloro-3-indoxylphosphate solution for about 15 min. at room temperature (for details see Blake et al., op. cit.).

### Example 4: Selection of Amino Acid Sequences Used for the Synthesis of Polypeptides Suitable for Diagnostic and Vaccine Purposes

For the synthesis of polypeptides we select several amino acid sequences out of the opacity proteins and class 5 proteins corresponding the following criterions:

Criterion (1): the polypeptide sequence have to be in accordance with the claims of this invention.

Criterion (2): the selected amino acid sequence should represent conserved antigenic determinant sites, which are mainly composed of hydrophilic amino acid residues (Fig. 2). Such conserved and hydrophilic amino acid sequences should be exposed on the surface of the gonococci and meningococci, respectively. Furthermore, such protein portions should fulfill general and important functions, for example as a binding site for receptors on mucosal cells. Such, probably immuno-recessive, epitopes of the opacity proteins and class 5 proteins should be rendered more immunogenic, when exposed as isolated polypeptides to the immune system of a patient.

Criterion (3): The DNA sequences coding for the selected polypeptides should be detectable in the genomes of all gonococcal and meningococcal strains and clinical isolates to ensure that the conservation criterion is not restricted to the strains investigated by DNA sequencing. A suitable test system therefor is a DNA dot blot assay using synthetic oligonucleotides as probes.

The polypeptide sequences selected by these criterions are shown in Table I.

Table I : Polypeptides corresponding to conserved and hydrophilic regions of the opacity and class 5 proteins.

POSITION	SEQUENCE (N-C)
38-50	STVSDYFRNIRTH
66-34	RIAADYARYRKWSDNKYSV*
210-230	YRYHNWGRLENTFRFKTHEASL

\* not tested in the dot blot assay (Criterion 3)

## Claims

1. A polypeptide which includes an amino acid sequence constituted by at least 5 and up to about 80 amino acid residues, and which is capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
2. The polypeptides in accordance with Claim 1 which include the amino acid sequences  
GRGPYVQADLAYAYEHITHDYP or  
GRGPYVQADLAYAYEHITRDYP or  
parts of said amino acid sequences, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
3. The polypeptide in accordance with Claim 2 which includes the amino acid sequence  
GRGPYVQADLAYAYEHIT or  
parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
4. The polypeptides in accordance with Claim 1 which include the amino acid sequences  
STVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKWSDNKYSV or  
STVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKWNDNKYSV or  
STVSDYFRNIRTHSIHPRVSVGYAFGGWRIAADYARYRKWNNNKYSV or  
STVSDYFRNIRTHSVHPRVSVGYDFGGWRIAADYARYRKWNNNKYSV or  
STVSDYFRNIRTHSIHPRVSVGYAFGGWRIAADYARYRKWHHNKYSV or  
STVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKWNNNKYSV or  
parts of said amino acid sequences, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
5. The polypeptide in accordance with claim 4 which includes the amino acid sequence  
STVSDYFRNIRT or  
parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
6. The polypeptide in accordance with Claim 4 which includes the amino acid sequence  
HPRVSVGY or  
parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
7. The polypeptide in accordance with Claim 4 which includes the amino acid sequence  
FGGWRIAADYARYRKW or  
parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
8. The polypeptide in accordance with Claim 4 which includes the amino acid sequence  
NKYSV or  
parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
9. The polypeptide in accordance with Claim 1 which includes the amino acid sequence  
SSLGLS or  
parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
10. The polypeptide in accordance with Claim 1 which includes the amino acid sequence  
FKPYIG or  
parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.

11. The polypeptide in accordance with Claim 1 which includes the amino acid sequence YGHVRH or parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
12. The polypeptides in accordance with Claim 1 which include the amino acid sequences  
 LTLDAGYRYHNWGRLENTFRFKTHEASLGVRVRF or  
 LTLDAGYRYHYWGRLENTFRFKTHEASLGVRVRF or  
 LTLDAGYRYHNWGRLENTFRFKTHEASLGMRYRF or  
 parts of said amino acid sequences, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
13. The polypeptide in accordance with Claim 12 which includes the amino acid sequence LTLDAGYRYH or parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
14. The polypeptide in accordance with Claim 12 which includes the amino acid sequence WGRLENTFRFKTHEASLG or parts of said amino acid sequence, as well as antigenically related variants of said polypeptides which are capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.
15. The polypeptides in accordance with Claims 2 to 14 having a proton at the amino terminus and a hydroxy group at the carboxy terminus, as well as a salt thereof.
16. A polypeptide containing up to about 2000 amino acid residues which consists of covalently linked multiple copies of at least one of the polypeptides according to anyone of claims 1 to 15 and optionally additionally of unrelated amino acid sequences.
17. A receptor molecule specifically binding to any of the polypeptides specified in Claims 1 to 16.
18. A receptor molecule raised in an animal host in accordance with Claim 17 wherein the receptor is a whole immunoglobuline.
19. A receptor molecule in accordance with Claim 17 wherein the receptor is an immunoglobuline combining site.
20. A diagnostic composition suitable for the detection of a gonococcal opacity protein (Protein II) and/or a meningococcal class 5 protein, comprising  
 (a) a receptor molecule according to Claims 17 to 19 and  
 (b) means capable of signalling an immune reaction of said receptor molecules with a gonococcal opacity protein (Protein II) and/or a meningococcal class 5 protein.
21. A diagnostic composition suitable for the detection of antibodies directed to a gonococcal opacity protein (Protein II) and/or a meningococcal class 5 protein in a sample, comprising  
 (a) a polypeptide according to any one of claims 1 to 16 and  
 (b) means capable of signalling an immune reaction of said polypeptide with said antibodies contained in the sample to be tested.
22. A vaccine composition suitable for the prevention of gonorrhea and/or meningitis comprising at least one polypeptide of any of Claims 1 to 16.
23. A genetically modified bacterial or viral system, which comprises a live vaccine suitable for the prevention of gonorrhea and/or meningitis, and which elicits at least one polypeptide of any of Claims 1 to 16.





Fig. 1 b

50	60	70	80
AGC GAT TAT TTC AGA AAC ATC CGT ACG CAT TCC ATC CAC CCC ACG GTG TCG GTT GGC TAC GAC TTC GGT GGC AGG ATA GCG GCA GAT TAT GCC CGT TAC AGG AAA TGG AGC GAC AAT			
S D Y F R N I R I H S I H P R V S V G Y D F G G W R I A A D Y A R Y R K W S D N			

..... AAC  
N

..... AAC AAC  
N N

..... AAC AAC  
N N

..... CAC AAC  
H N

..... AAC AAC  
N N

AGC GAT TAT TTC AGA AAC ATC CGT ACG CAT TCC ATC CAC CCC ACG GTG TCG GTT GGC TAC GAT TTC GGC GGC TGG AGG ATA GCG GCA GAT TAT GCC AGT TAC AGA AAA TGG AAA GAA ( )  
S D Y F R N I R A H S I H P R V S V G Y D F G G W R I A A D Y A S Y R K W K E

	90	100	110	120
AAA TAT TCC GTC	AGC ATA AAA AAT ATG CCG GTA CAT AAA CAC AAT AGC AAC AGG AAA CAC CTG AAG ACG GAA AAT CAG GAA AAC GGC AGC TTC CAC GGC GTT TCT TCT CTC GGC TTA TCC			

1331

.....GAC....CAG TTG GAA AAC ( ) AAG ( ) ... CAG AAT AAG ACA GAC ... ..GGT ACC ... ..GTC TCC TCG ... GGT TTG TCA  
D E L E N . Q K R D  
I

[illegible][illegible]

( ) ( ) AGT AAT TTT TCT ACT AAA AAA GTT ACT GAA GAG ATA AAA GAC AAC TAC AAA GAA ACC AAA ACA GAA CAT CAA GGA AAC GGC AGC TTC CAC GCC ACT TCT TCT CTC GGC TTA TCC  
 . . S H F S T K K V T E E I K D H Y K E T K T E H Q G W G S F H A T S S L G L S

Fig. 1 d

130 140 150 160  
 GCT ATT TAC GAT TTC CAA ATA AAC GAT AAA TTC AAA CCC TAT ATC GGC CCG CCC GTC GCC TAC GGA CAC GTC ACA CAC AGC ATC GAT TCG ACT AAA ATA ACA GCG GTT CTT ACC ACC  
 A I Y D F Q I H D K F K P Y I G A R V A Y G H V R H S I D S T K K I T G L L T I  
 ACC GTT ... AAA CTC ... GGT ... ACC ... ACA ... ANG YTT ... TEC  
 V K L T K F S  
 GCC GTT ... GAT ACC GGT TCC CGC ... ATG ... CAT CAG GTT CGT ... GTT CAA CAA GAA ACC ATT GCT GTT ... ACT  
 V D T G S R H Q V R V Q Q E I A V  
 ... CTA GAG GTT ACT ... L E V  
 ACC GTT ... ACA GTC ... GGT GTG CGT ... GGC ... GGT ... GGT ... ACC AAA AAT ACT ... GCC  
 T V R V V Q G T K H I A  
 GCC GTT ... GAT ACC GGT TCC CGC ... ATG ... CAT CAG GTT CGT ... GTT CAA CAA GAA ACC ATT GCT GTT ... ACT  
 V D T G S R H T V R V Q Q E I A V  
 GCC ATT TAC GAT TTC AAA CTC AAC GAT AAA TTC AAA CCC TAT ATC GGT GCG GTC GCC TAC GGG CAC GAT AAA CAT CAG GTT CAT TCG GTG GAA ACA AAA ACC ACG ACT GTT ACC TCT  
 A I Y D F K L N D K F K P Y I G A R V A Y G H V K H Q V H S V E T K T T V T S

Fig. 1 e

170 180 190 200  
 AGT ACT CCT GGC ATA ATG TCT GGG GTT TAT MAG GTA TTA AGG ACA CCA GGC GGC CAT CGC GAA AGC GAC AGC ATC CGC GCG GTG GGT CTC GGT ATC GGC GGC GTC GGT TTC GAC ATC  
 S T P G I M S G V Y K V L R T P G A H R E S D S I R R V G L G V I A G V G F D I  
 TCC TAT GGT ... TTA AAC CCT ACG ... ACT GAG GAA AAT ACG CAA AAC ... CAC CAA AGT AAC ... GGC ... GGC ...  
 Y G L N P T T E E N T Q N H Q N  
 TAC CCA CAG AAT GCT GCG TCA AGT ... ACC ACA AAT GCT CCG ATC CGC AAA CTT ... CAC ... CAC ... AGC AGC TTG GGC TTC GGC GCA GTG GCA ... GTA GGC ATC ...  
 Y P Q N A A S T T N A P I R K CCC  
 GTC CCC AGC AAT GCT CCT AAC GCA GCA GTT ACA ACT TAT AAT ACT GAT CCA AGG GAT TAC CAA ... AAC ... AAC ...  
 V P S N A P N A V T T Y N D P K D Y Q N  
 TAC CAT GGT GCT GGC ACA AAA CCT ACG ... TAT GAT GAT ATA GAT TCG GCA AAA TAT ... CAA AAC CGC ... AGC ... TTG GGC TTC GGC GCG ATG GCG ... GTG GGC ATA ... GTC  
 Y H G A G T K P T Y D D I D S G K Y Q N R S L F A H I V  
 TAC CCA CAG AAT GCT GCG TCA AGT ... ACC ACA AAT GCT CCG ATC CGC AAA CTT ... CAC ... CAC ... AGC ACG TTG GGC TTC GGC GCA GTG GCA ... GTA GGC ATC ...  
 Y P Q N A A S T T N A P I R K L H  
 AAA CCA AAG GGG GGC ACT CCA GCT GCA GGC CCT GTT ATA AAA ACT GAT CCC AGC TAT CAC GAA AGC CAC AGC ATC AGC AGC TTG GGT CTT GGT ATC GGC GGT GTC GGT TTC GAC ATC  
 K P K G G T P A G G P V I K I D P S Y H E S H S I S S L G L G V I A G V G F D I  
 AAA CCT CCC  
 K P P

Fig. 1 f

210										220										230																
ACG	CCC	ARG	CTG	ACC	CTG	GAC	GCC	TAC	CGC	TAC	CGC	TAC	CAC	AAC	TGG	GGA	CGC	TTG	GAA	AAC	ACC	CGC	TTT	AAA	ACC	CAC	GAA	GCC	TCA	TTG	GGC	GTG	CGC	TAC	CGC	TTT
T	P	K	L	T	L	D	A	G	-	Y	R	Y	H	N	W	G	R	L	E	N	T	R	F	K	T	H	E	A	S	L	G	V	R	Y	R	F

.....

.....

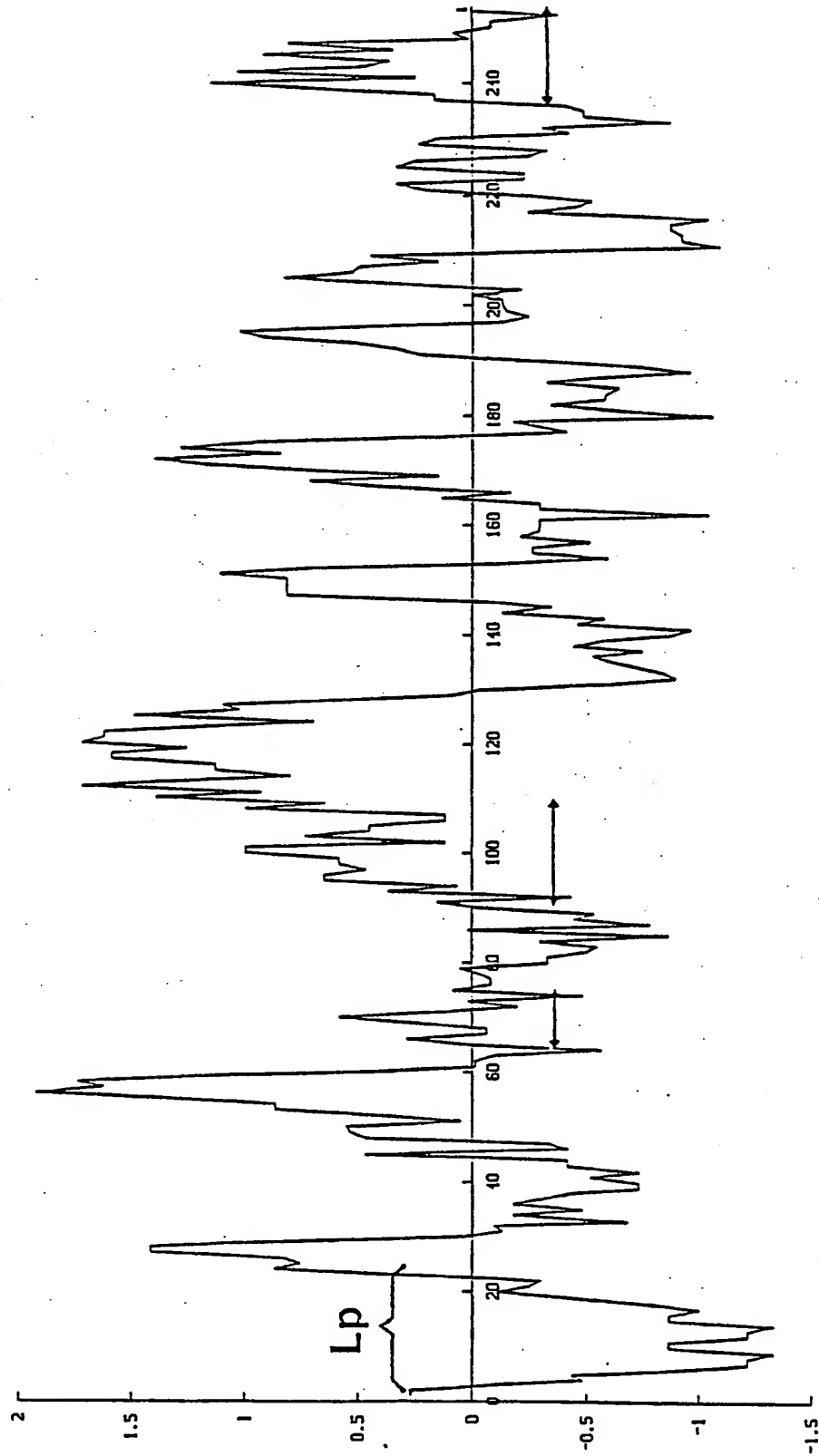
..... GGG TAT ..... AIG ..... H

GGG ... GGC ... TTG ... TAT ... CTG ...  
A G Y

..... AAC ..... TCG .....  
N

ACG CCC ARG CTG ACT TTA GAC ACC GGA TAC CGC TAC CAC AAC TGG GCA CGC TTG GAA AAC ACC CGC TTC AAA ACC ACC GAA GCC TCA TTG GGC ATG CGC TAC CGC TTC  
T P K L T L D T G Y R Y H N W G R L E H T R F K T Y E A S L G H R Y R F

Fig. 2



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(54) Gonococcal and meningococcal polypeptides, vaccines and diagnostics.

(57) The subject matter of the invention is a polypeptide which includes an amino acid residue sequence constituted by at least 5 and up to about 80 amino acid residues, and which is capable of immunologically mimicking a conserved antigenic determinant site of a gonococcal opacity protein (Protein II) and/or meningococcal class 5 protein.

The polypeptide of the invention can be used as a vaccine or diagnostic for the prevention of gonorrhea and/or meningitidis.

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. CL 4)
A	CHEMICAL ABSTRACTS, vol. 101, no. 11, 10th September 1984, page 135, abstract no. 84763r, Columbus, Ohio, US; A. STERN et al.: "Opacity determinants of Neisseria gonorrhoeae: gene expression and chromosomal linkage to the gonococcal pilus gene", & CELL (CAMBRIDGE, MASS.) 1984, 37(2), 447-56 * Abstract * ---	1	C 07 K 7/06 C 07 K 7/08 C 07 K 7/10 C 07 K 15/14 G 01 N 33/569 G 01 N 33/571 A 61 K 39/095 A 61 K 39/40 C 12 N 15/00 A 61 K 37/02
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A	BIOLOGICAL ABSTRACTS, vol. 80, no. 3, 1985, pages 394-395, abstract no. 58521, Biological Abstracts, Inc., Philadelphia, PA, US; D.S. STEPHENS et al.: "Analysis of structure and investigation of structural and antigenic relationships to gonococcal pill", & J. EXP. MED. 16(6): 1539-1553. 1985 * Abstract * --- -/-	1	TECHNICAL FIELDS SEARCHED (Int. CL 4)  C 07 K A 61 K G 01 N C 12 N
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CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	



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The present search report has been drawn up for all claims.			
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<b>CATEGORY OF CITED DOCUMENTS</b>			
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